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Tenascin Pattern of Expression and Established Prognostic Factors in Invasive Breast Carcinoma

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Background and Objectives: Immunohistochemical methods were used to study Tenascin (TN) expression in invasive duct cell carcinoma (IDCC) of the breast and its established prognostic factors.

Methods: We studied 115 patients with IDCC. The mean patient age was 62 years; all tumors were graded according to Scarf-Bloom and Richardson. Complete survival information was available for all patients (median follow-up 65 months). Formalin-fixed, paraffin-embedded archival tissue from all 115 IDCC were immunostained with monoclonal mouse Anti-Human Tenascin (DAKO-TN2M636; 1/50 dilution). Positivity was recorded on a scale of 0–4 for percentage of TN staining in the tumor stroma.

Results: TN showed thick bands around advancing tumor nests and in poorly differentiated tumors, TN fibers had an interstitial pattern surrounding single tumor cells. TN score was significantly positively correlated with high nuclear grade ($P < 0.05$), histologic grade ($P < 0.01$), mitotic grade ($P < 0.005$), and combined grade ($P < 0.01$). TN score did not correlate with long-term survival or with other prognostic factors studied.

Conclusions: TN expression was more prominent in tumors with a high combined histologic grade. Our results may suggest that while TN may play a role in limiting tumor spread as proposed by other studies, it may not represent a prognostic factor in invasive breast carcinoma.

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KEY WORDS: glycoprotein; extracellular matrix; adhesion molecule

INTRODUCTION

Tenascin (TN) is a glycoprotein component of the extracellular matrix (ECM). It was originally found in myotendinous junctions and named myotendinous antigen by Chiquet and Fambrough, in 1984 [1]. TN was initially thought to have a restricted expression during embryogenesis and oncogenesis [2]. Subsequently, TN was detected in various normal adult tissues, as well as in reparative and hyperplastic processes and in the stroma of a wide range of epithelial, mesenchymal, and glial tissues [3]. In carcinomas and in reparative and hyperplastic processes of epithelial type, TN expression is intense in the surrounding stromal fibroblasts, suggesting that it is most likely produced by fibroblasts and myofibroblasts. Also, TN was isolated from cultured fibroblasts [4].

The female breast frequently exhibits hyperplastic and neoplastic proliferation composed of glandular and stromal elements. In the mammary gland, TN was initially observed selectively in the condensing mesenchyme near budding epithelial cells of developing mammary glands, and also in the stroma of malignant tumors [2]. It was

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initially considered to be a stromal marker for mammary malignancy [5]. Later, TN was noted in the normal as well as hyperplastic adult breast. In the adult resting and aging breast, TN expression was noted in the periductal and periacinar stromal regions as thin, irregular bands. In hyperplasia, fibroadenomas and carcinoma in situ TN expression is increased forming thick periductal bands. In invasive breast carcinoma, TN expression is markedly increased in the stroma around infiltrating tumor cells [6].

The extracellular matrix of the breast has received comparatively little attention despite well-established data, suggesting that a dynamic cell matrix interplay is a prominent feature of the normal and pathologic breast [7]. The relationship between tumor invasion and TN expression is controversial. Although several studies have demonstrated that TN expression correlates with poor prognosis in epithelial tumors, other studies have demonstrated the opposite [8–12]. It is likely that TN may play a major role in the desmoplastic response of invading tumor cells. To better understand the role of TN in tumor invasion, we used immunohistochemical methods with archival tissue sections of formalin-fixed, paraffin-embedded surgical materials. In this study, we investigated the correlation of TN expression not only with long-term survival but also with recognized prognostic factors in 115 cases of invasive duct cell carcinoma of the breast.

MATERIALS AND METHODS

Patient Population and Inclusion Criteria

The records of 115 patients with histologically proven invasive duct cell carcinoma of the breast, stage I–III, were obtained from the tumor registry of Montefiore Medical Center, Bronx, NY. Only patients that were diagnosed between 1983–1986 were used for the study. Follow-up information, including survival and disease status, was known for all patients with a range of follow-up between 20–120 months (median 65 months). All of the patients underwent surgical resection of their tumors at Montefiore Medical Center. Hematoxylin and eosin stained slides from all included cases were reviewed by two of the authors. Each tumor was graded using the scheme of Scarf-Bloom-Richardson [13]. Accordingly, the nuclear grade, tubular grade, and mitotic index were determined for each tumor. The clinical and pathologic features of the patient population are shown in Table I.

TABLE I. Tumor Characteristics in 115 Cases of Invasive Duct Cell Carcinoma

Mean tumor size	3.1 cm (range 1.5–8.5 cm)
Combined histologic grade[a]	
Grade I (well differentiated)	4 (3%)
Grade II (moderately differentiated)	63 (55%)
Grade III (poorly differentiated)	48 (42%)
Lymph node status	
Positive	48 (42%)
Negative	55 (48%)
Unknown	12 (10%)
Estrogen receptors	
Positive	76 (66%)
Negative	31 (27%)
Unknown	8 (7%)
Progesterone receptors	
Positive	65 (57%)
Negative	39 (34%)
Unknown	13 (9%)

[a]Based on Scarf-Bloom-Richardson scheme [13].

TN was detected by immunohistochemical methods. The specimen blocks were retrieved, and two specimens were used as controls for localization and specificity of TN staining (normal breast tissue and adenomatous polyp). All specimens were cut into five micron sections and placed on lysine-coated glass slides. Slides were deparaffinized using xylene, and rehydrated with a graded ethanol series (100%–70%), followed by a rinse in water, incubation in 3% hydrogen peroxide for 20 min, washed in tap water, and pretreated with protease in phosphate-buffered solution (PBS) for 30 min at room temperature. The slides were rinsed in tap water, distilled water, and PBS. TN monoclonal mouse antihuman tenascin (DAKO, Carpinteria, CA. TN2M636; 1/50 dilution) was added and incubated 4 hr in a humidified chamber. Slides were washed in PBS and biotinylated antibody was added for 15 min, followed by rinse in PBS, and addition of streptavidin reagent for 15 min. After an additional PBS wash, the slides were treated with 3,3′-diaminobenzidine (DAKO, Carpinteria, CA) for 5 min. Slides were counterstained with hematoxylin, dehydrated, and mounted with glass coverslips.

TN positivity in tumor was graded from 0–4+ according to the total percentage of the tumor stroma that showed TN expression: 0–25% staining = 0–1+;25–50% staining = 2+: 50–75% staining = 3+: 75–100% staining = 4+. Intensity and patterns of staining in different tumor grades were also noted.

The relationship between TN score and established prognostic factors including tumor size, lymph node metastasis, hormone receptors, nuclear grade, mitotic index, histologic grade, and combined grades were analyzed using Spearman's correlation coefficient analysis [14]. The correlation between TN score and long-term survival were analyzed by univariate analysis, using the log rank test. *P*-values, based on two-tailed tests, were regarded as significant at less than 0.05.

RESULTS

Expression of TN in 115 cases of invasive duct cell carcinoma of the breast was studied by immunohistochemistry and graded on a scale from 0–4+. Expression of TN was found as a thin band around normal ducts;

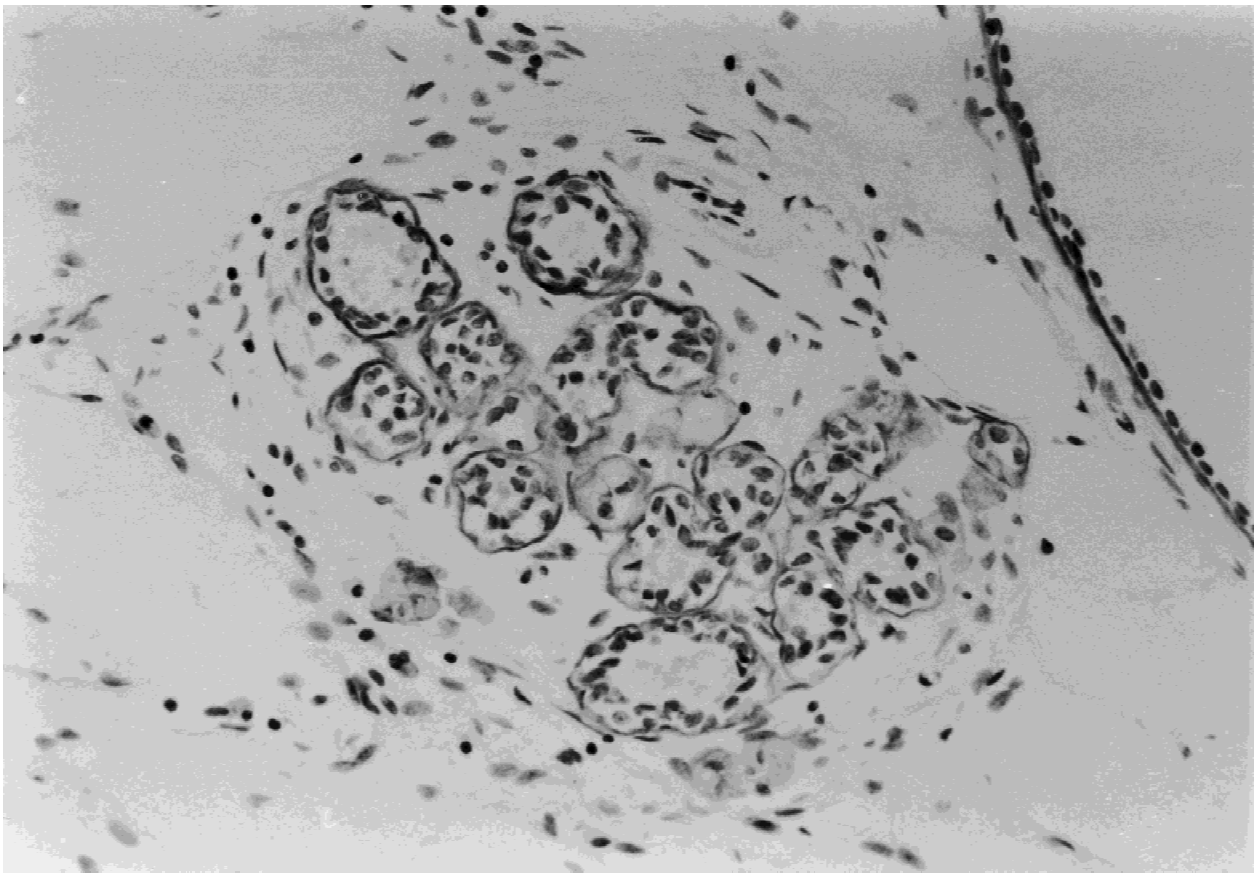

Fig. 1. Tenascin expression showing periductal staining forming thin bands in normal breast tissue (×100).

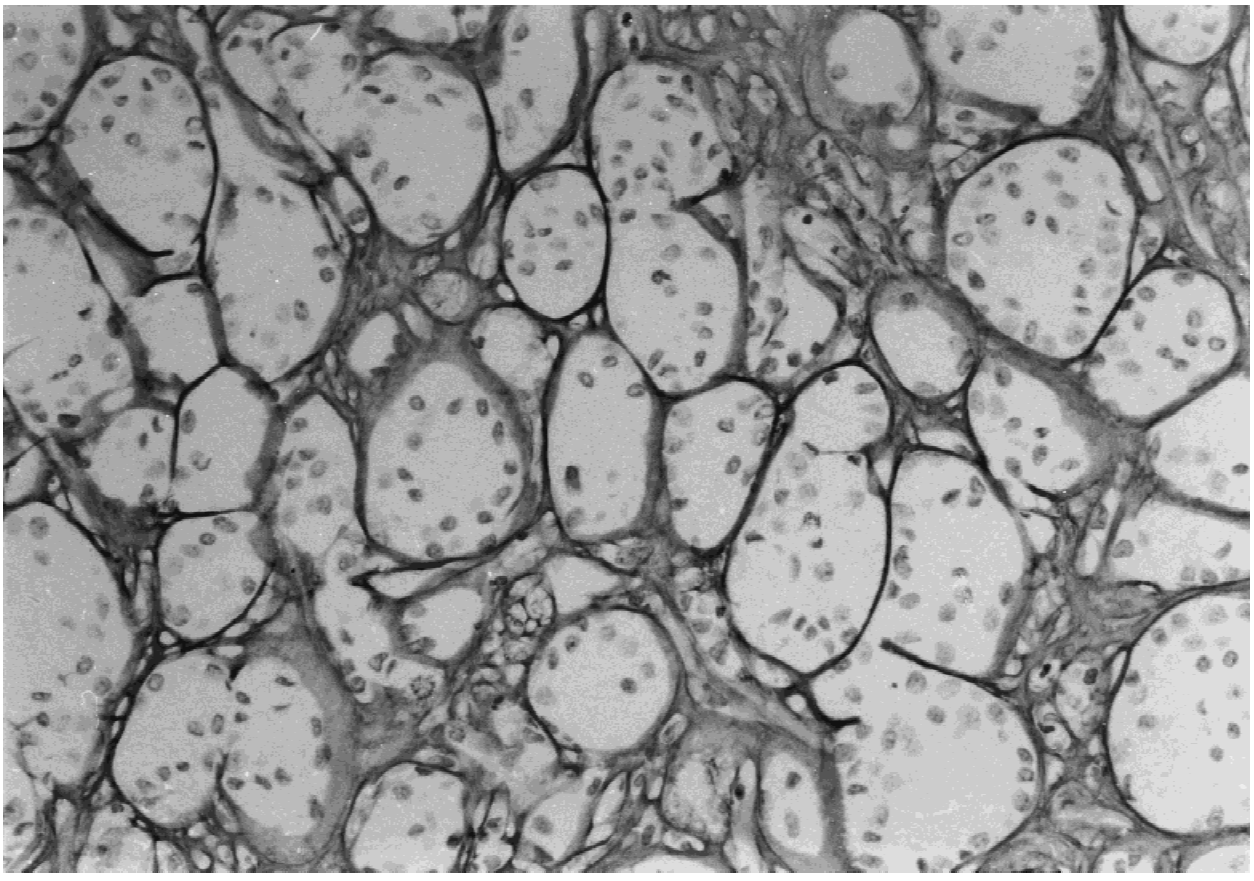

Fig. 2. Tenascin expression showing staining forming thick bands around invasive tumor nests in well-differentiated carcinoma (×200).

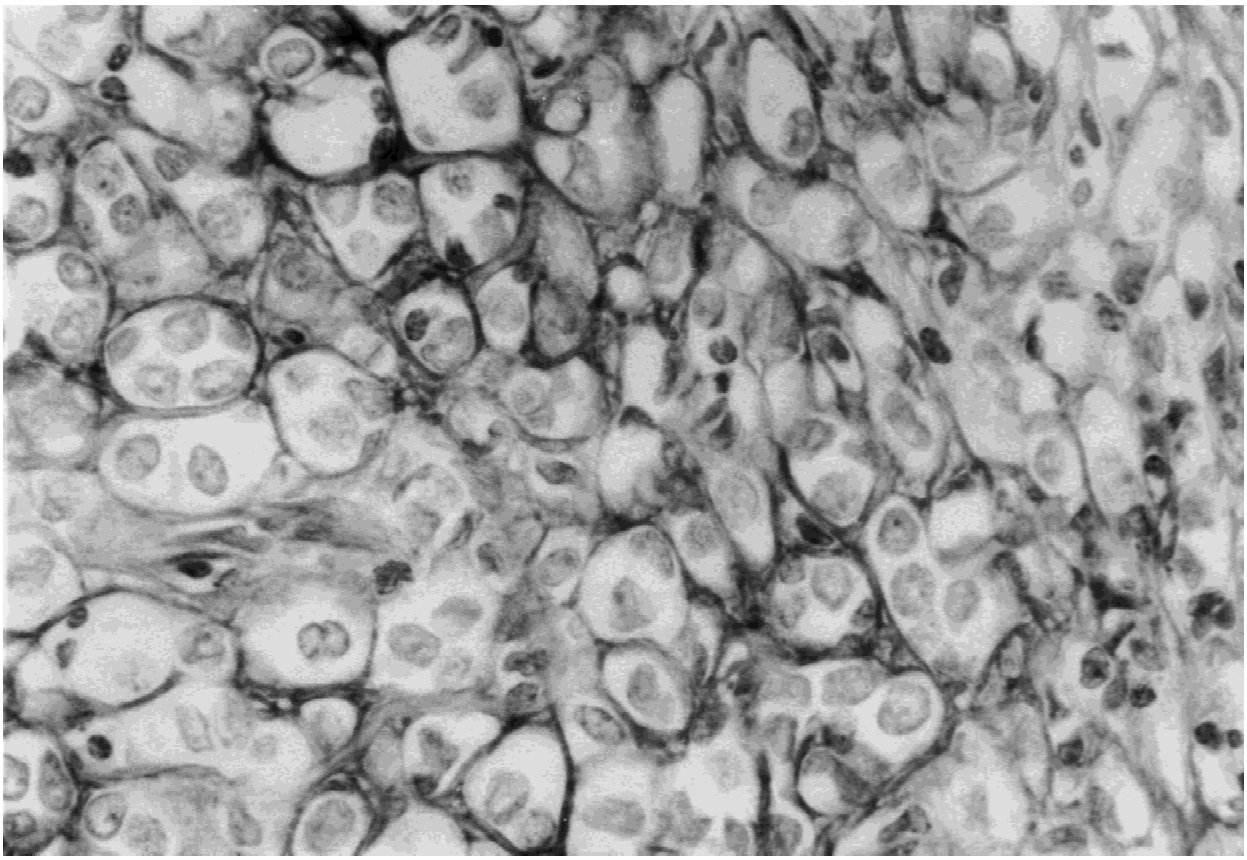

Fig. 3. Tenascin expression showing interstitial pattern of staining (fish-net) network around individual and small groups of tumor cells in poorly differentiated carcinoma (×400).

whereas stroma infiltrated by tumor showed strong TN staining, while tumor cells did not show any staining (Figs. 1,2). In well-differentiated tumors, TN expression was seen as thick bands in the stroma surrounding invading tumor ducts and nests (Fig. 2). In poorly differentiated tumors, TN expression was diffuse and interstitial, forming a ''fish-net'' network of fibers around individual and small groups of tumor cells (Fig. 3). TN expression was also consistently found surrounding blood vessel walls in the vicinity of the infiltrating tumor (Fig. 4). In contrast, in normal breast tissue, blood vessels were negative for TN.

Spearman's correlation coefficient analysis showed that high TN score was significantly positively correlated with high nuclear grade ($P < 0.05$), mitotic index ($P < 0.005$), and combined grade ($P < 0.01$) as shown in Figures 5–7. Other prognostic factors, including tumor size, lymph node status, and hormone receptors, did not show a correlation with TN score. No correlation was found between TN score and long-term survival.

DISCUSSION

TN has been reported to have a wide spectrum of functions. These include cell adhesion/anti-adhesion, neural crest cell migration/regulation, and cancer cell growth/inhibition [15–17]. Immunohistochemistry for TN in various tissues clearly shows that it appears in the basement membrane and extra cellular space when the tissue is undergoing rearrangement and remodeling. The role of TN in tumorogenesis is controversial. One possible approach to the elucidation of the TN function is the study of what induces its production in the tissue. Transforming growth factor beta (TGF-β) is a regulatory protein that can stimulate the production of extracellular matrix proteins [18–21]. The conditioned medium of human breast cancer cell lines MCF7 do not produce TN in vitro, but produce TN when co-cultured with chick embryo fibroblasts. This activity of the conditioned medium was neutralized by anti-TGF-β antibody, and MCF7 is known to secrete biologically active TGF-β [22]. Therefore, it can be speculated that one possible factor that manages the epithelial–mesenchymal interaction is TGF-β.

Our data shows increased TN expression around blood vessel walls and the stroma of higher grade tumors ($P < 0.01$). This may explain the role of TN and its increasing expression in response to more aggressive tumors in a way to limit their progression and spread to distant sites.

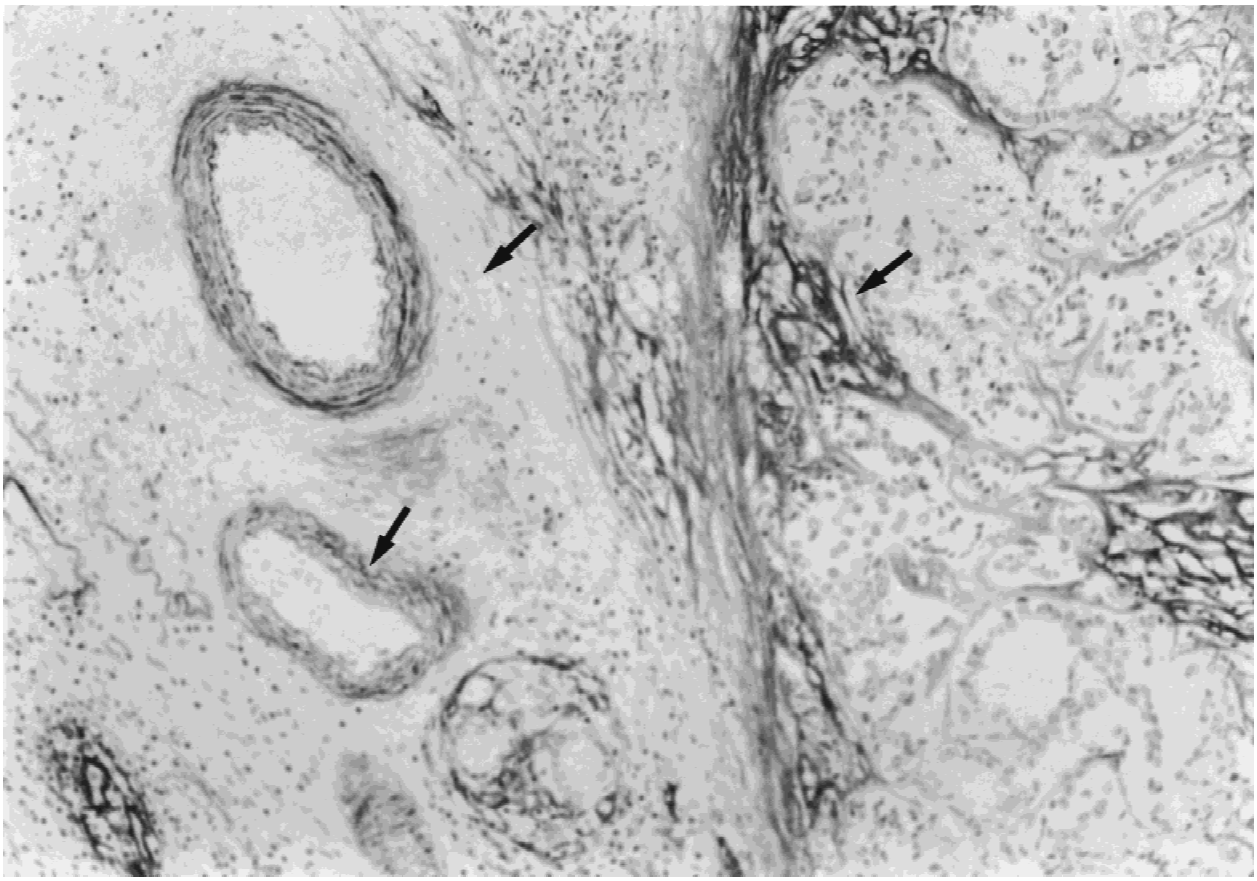

Fig. 4. Tenascin expression at the blood vessel walls and stroma (arrows) in the vicinity of the infiltrating tumor (×100).

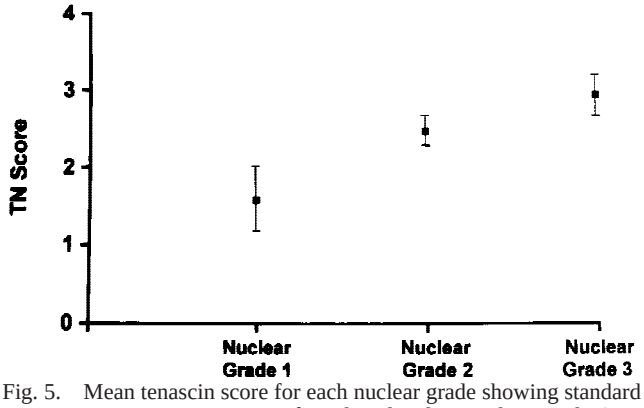

Fig. 5. Mean tenascin score for each nuclear grade showing standard error. Tenascin score was significantly related to nuclear grade ($P < 0.05$).

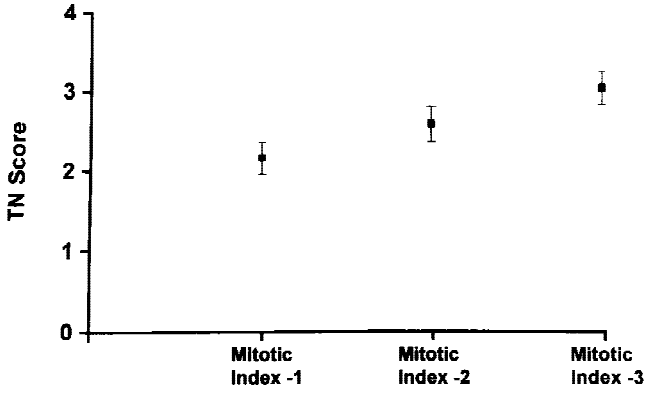

Fig. 6. Mean tenascin score for each mitotic index showing standard error. Tenascin score was significantly related to mitotic index ($P < 0.005$).

This theory is supported by previous reports of better prognosis in TN positive tumors [10–12]; however, in our study no correlation was found between TN score and long-term survival.

TN was believed to have been produced only by mesenchymal cells. Recent studies demonstrated that cancer cells also produce TN in culture or in growing tumor when injected in vivo [23–25]. Immunohistochemistry of cancer tissues, including comedo carcinoma of the breast [7] and squamous cell carcinoma and adenocarcinoma of the lung [26], have demonstrated cytoplasmic TN staining, suggesting that cancer cells also produce TN. Furthermore, in situ hybridization of freshly obtained breast cancer tissues demonstrated that both cancer cells and stromal cells express TN in mRNA [27,28]. In malignant tumors, it is likely that both stromal cells and cancer cells can produce TN at the same time [23]. There are con-

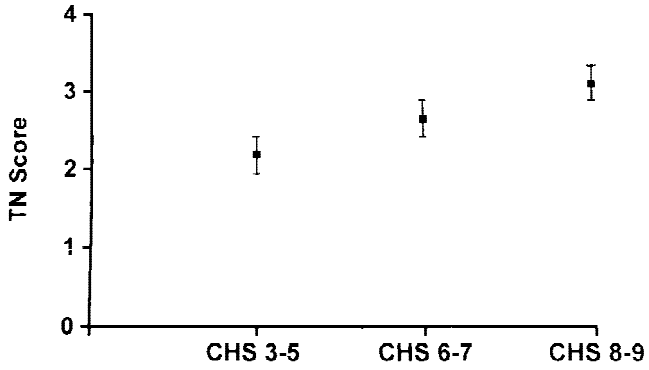

Fig. 7. Mean tenascin score for each of 3 groups of combined histologic score (CHS) showing standard error. Tenascin score was significantly related to CHS ($P < 0.01$).

flicting reports [8–12] on TN and prognosis, including one report on epithelial TN as a marker for poor prognosis [8]. Since TN acts as an anti-adhesion molecule, it could help both stromal and epithelial cells to detach from their substratum. This leads us to speculate that cancer cells may produce TN, making the tissue loosely bounded and allowing cancer cells to migrate and metastasize easily. On the other hand, stromal cells may produce TN which functions in different way. Favorable function of stromal TN would be that it helps create boundaries around cancer cells, inhibits cancer cell migration, and regulates cancer cell growth.

In our study TN expression did not show significant correlation with other prognostic variables including tumor size, lymph node status, and hormone receptors. These findings require a larger study of patients for understanding the role of TN in tumor invasion and metastasis and its correlation with prognosis.

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